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# The Effect of Drought Stress on Germination characteristics and Proline Changes in Two Cultivars of Alfalfa

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**ABSTRACT**

One of the effects of water depletion in the soil is the reduction of seedlings growth and development and changes in their field development under such conditions. In order to investigate the effect of drought stress and cultivar on germination indices and alfalfa proline changes in Petri dish and Pot conditions, a factorial experiment as randomized complete block design with 4 replications was carried out in the specialized laboratory of agronomy at Ahvaz Azad University. In both Petri dish and Pot culture conditions the first factor included Yazdi and Baghdadi cultivars of alfalfa and the second factor included 5 levels of drought stress (0, 2, 4, 6, 8 bars). The results showed that the interactive effect of cultivar and drought stress on all the measured traits were significant. Drought stress reduced germination indices. The highest germination percentage in both Petri dish and pot conditions by 99 and 91% respectively belonged to Yazdi cultivar and without stress. In general, the highest rate of all measured traits was related to Yazdi cultivar and absence of stress. Moreover, the results showed that as the drought stress levels increased the rate of proline increased particularly in Yazdi cultivar. At the highest levels of drought stress the highest germination characteristics belonged to Yazdi cultivar. Therefore, it can be said that under drought stress conditions it is better to use Yazdi cultivar.



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## INTRODUCTION

Drought and the stress resulted from it are among the most important and the most common environmental stresses which have limited agricultural productions and have reduced the efficiency of using semi-arid areas [18].

Germination is the first growth stage in plants and is a major and critical stage in the life cycle of plants and a key process in seedling growing [30]. Inappropriate environmental conditions for germination and seedling emergence in arid and semi-arid areas are the major causes of poor emergence and weak establishment of seedlings. Environmental stresses such as drought stress will reduce germination characteristics and emergence uniformity. Reduction of germination due to drought stress can be associated with the decrease of water absorption by seeds [4]. Different studies have shown that germination and seedling growth will reduce under the effect of various abiotic stresses such as salinity, drought, and the cold [19, 22, 4]. Studying the effect of drought stress on annual alfalfa at germination stage showed that germination index decreased as the osmotic stress increased [24]. Studying the effect of polyethylene glycol on germination of different species of alfalfa, the researchers showed that as the osmotic potential increased, germination indices decreased [16].

At environmental stresses such as drought, salinity, heat, etc plants cope with stresses by accumulation of osmotic regulators [7]. Proline is an amino acid dissolved in water whose amount increases in environmental stress conditions and its increase indicates plant resistance against stress conditions. Proline keeps proteins and cell membranes from the damage caused by high concentrations of ions [23]. Safar Nejad *et al.* [26] examined the effect of osmotic stress on alfalfa genotypes and reported that resistant genotypes showed greater and faster reaction than the susceptible species in terms of proline accumulation. Proline accumulation during the drought stress has been reported in other plants such as Peas [13], Wheat [20], Maize [27] and Peanut [29], too.

Alfalfa with the scientific name of Medicago Sativa is the most important forage in the world which grows everywhere except in tropical areas [17]. Due to lack of detailed knowledge of the behavior of forage species

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against drought stress and their resistance to the drought stress it is necessary to do extensive studies in this regard, so that it could be possible through better knowledge to select species which are resistant to drought stress in creating germination in arid and semiarid areas.

Therefore, this research was carried out to investigate the effect of drought stress on germination components and seedlings proline changes in two alfalfa cultivars.

## MATERIALS AND METHODS

In order to investigate the effect of drought stress on germination characteristics and proline changes in two Baghdadi and Yazdi cultivars of alfalfa, separate factorial experiments as randomized complete block designs with 4 replications were carried out in the specialized laboratory of agronomy at Ahvaz Azad University.

*Culture in Petri Dishes:*

The experiment was carried out on different cultivars of alfalfa by creating drought stress at five levels with osmotic pressures of 0, 2, 4, 6, 8 bars (Michelle Kaufmann method) and by using PEG (Polyethylene Glycol 6000). First, the seeds were disinfected with Sodium Hypo Chloride 3% for two minutes, then they were washed three times with distilled water and 50 seeds were transferred to 10-cm-diameter glass Petri for drought stress and 6 ml polyethylene glycol 6000 (Michelle Kaufmann) during the experiment period was added to Petri dishes with different levels at temperature of 20 ±1°C and they were taken to darkness conditions until the germination didn't occur anymore. The number of germinated seeds was counted and recorded every day.

After the end of germination period, the following indices were measured: germination percentage, germination rate, mean time to germination, seedling dry weight, and seedling fresh weight.

*Culture in Pots:*

For cultivation in pots, small pots with diameter of 10 cm and height of 20 cm were used. First, the pots were filled with washed sands and then 5 disinfected seeds were placed in each pot and were covered by sand. Afterward, osmotic solutions were created by polyethylene glycol 6000 (industrial polyethylene glycol) and added to the pots. The applied levels of stress included 0, 2, 4, 6, and 8 bars. After sometime when germination didn't occur anymore in two days, the germination percentage was evaluated.

The seedlings were taken out of the pots and the length and dry weight of them were measured. Finally, the samples were selected and used for investigating proline changes.

*Measurement of Proline:*

After the completion of germination period plant leaves were harvested and proline content in plant tissues was measured using the approach of Betis *et al.* [9]. Proline concentration in milligram per gram (mg/g) of fresh leaf tissue was determined by using a standard curve.

Data analysis was done by MSTAT-C software and the means were compared to each other by means of minimum significant difference (Duncan) test. The diagrams were drawn by Excel software.

## RESULTS AND DISCUSSION

The ANOVA results of the effect of drought stress on germination characteristics of two alfalfa cultivars under cultivation conditions in Petri dishes showed that the effect of cultivar on germination percentage, germination index, mean time to germination and seedling dry weight were significant at 1% level and on seedling fresh weight was significant at 5% level, and the effect of drought stress on all traits were significant at 1% level (Table 1). The effects of cultivar × stress on all traits except seedling fresh weight were significant.

**Table 1:** The ANOVA results of the effect of drought stress on germination characteristics in two alfalfa cultivars under cultivation conditions in Petri dishes.

| S.O.V | df | Germination percentage | Germination index | Mean time to germination | Seedling fresh weight | Seedling dry weight |
|---|---|---|---|---|---|---|
| r | 3 | $54/8^{ns}$ | $3/62^{ns}$ | $0/09^{ns}$ | $0/003^{ns}$ | $0/000002^{ns}$ |
| Cultivar | 1 | $608/4^{**}$ | $251/62^{**}$ | $4/61^{**}$ | $0/02^{*}$ | $0/0001^{**}$ |
| Stress | 4 | $4536/4^{**}$ | $432/58^{**}$ | $37/89^{**}$ | $0/11^{**}$ | $0/0003^{**}$ |
| Cultivar × Stress | 4 | $66/4^{*}$ | $59/88^{**}$ | $0/32^{**}$ | $0/004^{ns}$ | $0/00003^{**}$ |
| Error | 27 | 23/69 | 1/41 | 0/03 | 0/004 | 0/000001 |
| CV % | - | 6/33 | 10/53 | 5/9 | 21/11 | 6/47 |

**, *, ns respectively indicate significant at 1% and 5% levels and non-significant.

The ANOVA results of the effect of cultivar and drought stress on germination characteristics of alfalfa seeds under cultivation conditions in pots showed that the effect of cultivar × drought stress on all traits were significant at 5% level (Table 2).

**Table 2:** The ANOVA results of the effect of cultivar and stress on germination characteristics of alfalfa seeds under cultivation conditions in pots.

| S.O.V | df | Germination percentage | Seedling lenght | Seedling dry weight | Proline |
|---|---|---|---|---|---|
| r | 3 | $14/39^{ns}$ | $3/62^{ns}$ | $0/000004^{ns}$ | $0/00002^{ns}$ |
| Cultivar | 1 | $140/17^{**}$ | $3/03^{**}$ | $0/001^{**}$ | $0/001^{**}$ |
| Stress | 4 | $3579/17^{**}$ | $73/9^{**}$ | $0/0004^{**}$ | $0/01^{**}$ |
| Cultivar × Stress | 4 | $22/17^{*}$ | $0/6^{*}$ | $0/00002^{*}$ | $0/00005^{*}$ |
| Error | 27 | 5/72 | 0/15 | 0/000004 | 0/00001 |
| CV % | - | 3/34 | 6/04 | 7/72 | 6/77 |

**, *, ns respectively indicate significant at 1% and 5% levels and non-significant.

Mean comparison of the interactive effect of cultivar and drought stress on germination percentage, germination index, and dry weight under cultivation conditions in Petri dishes and pots showed that the highest percentage of germination by 99% and 91% belonged to cultivation conditions in Petri dishes and pots respectively. The highest germination index by 75.24 seeds per day and the highest dry weight and seedling length belonged to Yazdi cultivar and lack of stress (Table 3, 4). As drought stress increased, germination percentage, germination index, dry weight, and seedling length decreased (Table 3, 4). The findings of different researchers on different plants confirmed the results of the experiment [5, 3, 1]. It has been found that the seeds of genotypes which were more tolerant of water stress at germination and seedling stages had the same ability in later stages, as well [10]. The decrease of germination index has been attributed to the decrease of water absorption and germination rate [4, 1, 28]. In cultivation condition in pots the seeds didn't show germination under stress conditions of 6 bars and the dry weight was measured up to the stress level of 4 bars. Hamidi and Safar Nejad [16], Mahmoudi *et al.* [21] and Reshno [24] studied alfalfa and reported that the increase of drought stress levels significantly decreased the seedling dry weight and length. Ansari *et al.* [4], Soltani *et al.* [30], Das *et al.* [11] and Gupta *et al.* [15] reported that the decrease of seedling dry weight at high levels of drought stress was due to the decrease of transfer of nutrients from cotyledons or endosperms into the embryonic axis and there was a direct relationship between dry matter accumulation and seedling growth in tolerant plants.

The results showed that as the stress levels increased the mean time to germination increased and the highest mean time to germination belonged to stress level of 8 bars and Baghdadi cultivar and the lowest average time belonged to Yazdi cultivar and stress levels of 0 and 2 bars (Table 3, 4). Various researchers have shown that drought stress increases the mean time to germination which could be due to the decrease of water absorption and germination rate [1, 22].

**Table 3:** The effect of drought stress on germination characteristics in two alfalfa cultivars under cultivation conditions in Petri dishes.

| Cultivar | Drought stress (bar) | Germination percentage | Germination index | Mean time to germination | Seedling dry weight (gr) |
|---|---|---|---|---|---|
| Yazdi | 0 | $99^{a}$ | $24.75^{a}$ | $1^{a}$ | $0.031^{a}$ |
| | 2- | $93^{ab}$ | $23.12^{a}$ | $1.01^{g}$ | $0.028^{bc}$ |
| | 4- | $89^{ab}$ | $10.95^{c}$ | $2.1^{ef}$ | $0.024^{bc}$ |
| | 6- | $77^{cd}$ | $8.02^{d}$ | $2.67^{cd}$ | $0.017^{cd}$ |
| | 8- | $46^{e}$ | $1.99^{e}$ | $6.28^{b}$ | $0.008^{de}$ |
| Baghdadi | 0 | $95^{ab}$ | $14.73^{b}$ | $1.91^{f}$ | $0.029^{ab}$ |
| | 2- | $87^{bc}$ | $11.21^{c}$ | $2.11^{ef}$ | $0.024^{bc}$ |
| | 4- | $84^{bc}$ | $9.64^{cd}$ | $2.39^{de}$ | $0.018^{bc}$ |
| | 6- | $71^{d}$ | $7.15^{d}$ | $2.87^{c}$ | $0.012^{bc}$ |
| | 8- | $28^{f}$ | $1.02^{e}$ | $7.16^{a}$ | $0.005^{e}$ |

Means with similar letters in each column are not significantly different according to least significant difference test at the 5% level.

Mean comparison results of the effect of drought stress on seedling fresh weight showed that as the drought stress increased seedling fresh weight significantly decreased. The highest fresh weight belonged to the treatment without stress and the lowest fresh weight belonged to stress level of 8 bars (Fige 1).

Mean comparison results of simple effect of cultivar on fresh weight showed that the highest weight belonged to Yazdi cultivar (Fig 2).

Mean comparison results of simple effect of cultivar and drought stress on proline content showed that as the drought stress increased proline content increased, too. The highest rate of proline content was related to Yazdi cultivar at stress level of 4 bars (Table 4). Higher rate of proline content in Yazdi cultivar could result in greater resistance of Yazdi cultivar. Therefore, the increase of proline content at high levels of stress in Yazdi cultivar leads to the resistance of this cultivar to stress conditions.

Means with similar letters in each column are not significantly different according to least significant difference test at the 5% level.

Safar Nejad *et al.* [25] investigated the effect of osmotic stress on alfalfa genotypes and reported that resistant genotypes show more and faster reactions than susceptible species in terms of proline accumulation,

i.e. resistant species have more proline accumulation at high levels of stress [26, 14]. Proline accumulation during drought stress has been reported in other plants, as well [25, 27].

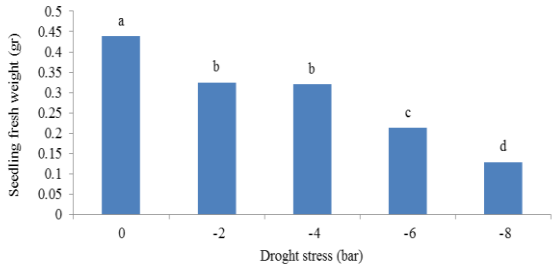


**Fig. 1:** The effect of drought stress on seedling fresh weight.

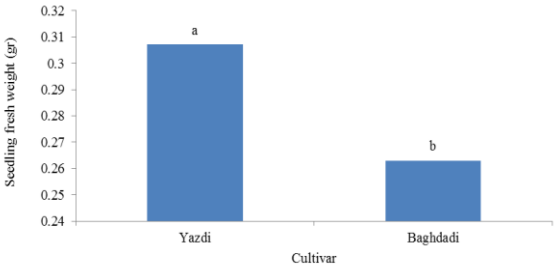


**Fig. 2:** The effect of Cultivar on seedling fresh weight.

**Table 4:** The effect of drought stress on germination characteristics in two alfalfa cultivars under cultivation conditions in pots.

| Cultivar | Drought stress (bar) | Germination percentage | Seedling lenght | Seedling dry weight | Proline (mg/gr fw) |
|---|---|---|---|---|---|
| | 0 | $90.5^{a}$ | $9.7^{a}$ | $0.04^{a}$ | $0.034^{a}$ |
| | 2- | $79^{b}$ | $6.41^{b}$ | $0.03^{b}$ | $0.049^{c}$ |
| Yazdi | 4- | $52.5^{c}$ | $3.99^{c}$ | $0.024^{c}$ | $0.089^{a}$ |
| | 0 | $88.5^{a}$ | $9.13^{a}$ | $0.04^{a}$ | $0.029^{d}$ |
| | 2- | $75^{b}$ | $6.16^{b}$ | $0.02^{cd}$ | $0.036^{d}$ |
| Baghdadi | 4- | $44^{d}$ | $2.68^{d}$ | $0.017^{d}$ | $0.076^{b}$ |

Drought stress decreases the seed germination by limiting the available water for the seed and thus reduces germination indices. Plants adopt various strategies to cope with drought stress. One of the strategies adopted by plants to deal with environmental stress is to increase proline content. The more the plant increases its proline content the more efficient it will be to deal with stress; however, it should be noted that the increase of proline content is useful up to the stress tolerance threshold and at very high stress levels the plant will die.

*Conclusion:*

The results of the experiment showed that drought stress led to the decrease of germination indices in both alfalfa cultivars (Yazdi and Baghdadi). There is a relationship between germination indices and stress tolerance in different cultivars, so that Yazdi cultivar which had higher germination indices than Baghdadi cultivar was more tolerant of stress. As drought stress increased, proline content increased and the increase in Yazdi cultivar was more than Baghdadi cultivar.

In general, any cultivar among different cultivars which has higher germination indices particularly germination percentage, germination rate, seedling length, and proline content is more tolerant of unfavorable environmental conditions. In this experiment, Yazdi cultivar had such characteristics.

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